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(54) THERAPEUTIC AGENT FOR NEOVASCULAR DISEASE

(57) Abstract:

PROBLEM TO BE SOLVED: To obtain the subject therapeutic agent containing specific lactoferrins (related substance) as active ingredients, free from adverse effect, having high safety and useful for treating ophthalmological disease group, chronic rheumatoid arthritis, psoriasis, abnormal capillary vasoganglion of atheromatous arteriosclerosis nidal adventitia.

SOLUTION: This therapeutic agent contains lactoferrins (A1), a hydrolyzate of A1 (A2), a peptide (A3) derived from A2, a peptide (A4) having an amino acid sequence which is same as or similar to that of the peptide (A3), a derivative of these peptides, a salt of these peptides or a mixture thereof as an active ingredient, e.g. in an amount of 0.1μg to 100mg based on 1g preparation. Furthermore, the peptide as the active ingredient is preferably composed of an amino acid sequence expressed by formulae I to a III (ROI is an amino acid residue other than Cys), etc., or constituted so as to contain the sequence as a fragment.

Lys ROI RO. ROI ROI Gln ROI ROI Met Lys Lys
1 5 10

I

Lys ROI RO. ROI ROI Gln ROI ROI Met Arg Lys
1 5 10

II

Arg ROI ROI ROI ROI Arg

III

* NOTICES *

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The hydrolyzate of lactoferrin and lactoferrin, the peptide of the hydrolyzate origin of lactoferrin, the same or derivative of the peptides which have the amino acid sequence of homology, and these peptides as this peptide permitted pharmacologically, the salts of these peptides permitted pharmacologically, or the blood vessel new disease medical treatment agent which contains such mixture as an active principle.
[Claim 2] The vascularization disease treatment agent of the claim 1 whose content of an active principle is 0.1micro per 1g of tablets g-100mg.
[Claim 3] The vascularization disease treatment agent of the claim 1 whose peptide is a peptide which has the amino acid sequence indicated by either of the array number 1 to the array numbers 31, or a claim 2.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] This invention relates to the therapeutic drug agent for vascularization diseases, such as an unusual capillary network of the ophthalmology-disorder group which is an intractable disorder, rheumatoid arthritis, psoriasis, and an atherosclerosis nest outer membrane.
[0002]
[Description of the Prior Art] The vascularization is the phenomenon in which an endothelial cell comes into bud from a vessel, and the new vasoganglion is formed. The process is classified into envelopment of the endothelial cell by the lumen formation by destruction of the basement membrane by ** protease, germination of dissolution and ** endothelial cell, and division proliferation of migration and ** endothelial cell and specialization of ** endothelial cell, formation of ** basement membrane, and ** pericyte (the experimental medicine, the 10th volume, the 48th page, 1992).
[0003] In recent years, the concept of a vascularization disease (angiogenic diseases) was advocated by fall KUMAN (Folkman) and crag SUBURUN (Klagsbrun) about the disorder which makes unusual neogenesis of a vessel symptoms [a science (Science), the 235th volume, the 442nd page, and 1987]. That is, in the various disorders currently considered to be till then completely unrelated, since it became clear that fundamental symptoms are the plagues of a capillary, it came to name such a disorder group a vascularization disease generically.
[0004] According to Ito (metabolism, the 25th volume, No. 12, the 1075-1081st page, 1990), this vascularization disease is defined as the general term of the following disorder group.
1) Solid neoplasm 2 ophthalmology-disorder ** proliferative-diabetic-retinopathy ** retinopathy of prematurity (back lens fibroplasia)
** As the therapeutic drug of the unusual capillary network former of a symptoms 3 rheumatoid-arthritis 4 hemangioma [which causes iris rubeosis ** sickle-cell-retinopathy ** vena-centralis-retinae atresia ** retinal-vein branching atresia ** central-artery-of-retina atresia ** senile-disciform-macular-degeneration ** and other ****], and hemangiofibroma 5 psoriasis 6 atherosclerosis nest outer membrane, and these vascularization disease. combined use [of ** a protamine a steroid combined use of a heparin and a steroid, combined use of hex RONIRU HEKISOSAMINO glycan sulfate and mitoxantrone the heparin joint fragment of a fibronectin, a prostagladin synthetase inhibitor, a gamma interferon, a gold compound, lymphotoxin, D-penicillamine, a steroid, and beta-cyclodextrin tetrapod cuttlefish sulfate] protease inhibitor, methotrexate, and interferon alpha 2a etc. -- it is known (metabolism, the 25th volume, No. 12, the 1075-1081st page, 1990)
[0005] On the other hand, lactoferrin (it may be indicated as lactoferrin and Following Lf) is iron unity glycoprotein of the molecular weight 80,000 [about] contained very so much in mother's milk. It is known that an antibacterial action is shown to detrimental microorganisms, such as Escherichia coli, the Candida bacillus, the Clostridium bacillus, and staphylococcus, [journal OBU PEDIATORIKUSU (Journal of Pediatrics), the 94th volume, the 1st page, 1979 and a journal OBU daily science (Journal of Dairy Science), the 67th volume, the 60th page, and 1984]. Moreover, it has proved that the effect of Lf is also reported using the infection model animal, and compare a survival

rate about the group which prescribed Lf for the patient into the vein 24 hours before ZAGURUSUKI and others (Zagulski) prescribed the Escherichia coli of a lethal dose for the patient using the rat, and the group non-prescribed a medicine for the patient, and Lf has a phylaxis operation [British journal OBU experimental PASOROJI (British Journal of Experimantal Pathology), the 70th volume, the 697th page, and 1989].
[0006] [the cancer research (Cancer Research), the 47th volume, the 4184th page, and 1987] when, as for a certain thing, the phylaxis effect is known by Lf also in the viral infection experiment [furthermore,] It is thought that it is because Lf carried out activation of a host's immunity force rather than it thinks that the effect accepted for these infection animals is based on the antibacterial action accepted by in vitro (inside of a test tube) of Lf. That is, it is understood by Lf that there is an immunity activation operation other than an antibacterial action. There is also an example which applied the immunity activation operation of Lf to anti-cancer, and it is BEZAURUTO and others (Bezault). [the cancer research (Cancer Research), the 54th volume, the 2310th page, and 1994] considered the effect which medicates a cancer model mouse with Lf into a vein, and examines the effect of Lf to growth and transition of cancer, consequently suppresses growth and transition of cancer to Lf is accepted, and are especially based on the activation operation which is a spontaneous killer cell from a viewpoint of an immunity activation operation
[0007] Although vascularization prevention activity is from a different viewpoint, the antitumor agent (JP,5-85932,B) and the antirheumatic (JP,5-186368,A) are known as an example which applied lactoferrin to the treatment agent of the disorder applicable to the vascularization disease by aforementioned Ito. Moreover, about the decomposition product of Lf, antibacterial and tyrosinase activity prevention (European Patent public presentation No. 438750), disease germ antisticking (JP,3-220130,A) to a cell, the antiviral action (JP,1-233226,A), etc. are known. Furthermore, the antitumor agent for parenteral (JP,7-309771,A) which makes an active principle two or more sorts of peptide mixture which has the specific amino acid sequence acquired from the hydrolyzate of lactoferrin is also known.
[0008]
[Problem(s) to be Solved by the Invention] Although various vascularization disease treatment agents were developed from before as aforementioned, the thing of those many was a thing of a chemosynthesis article and the microorganism origin etc., and was not necessarily desirable about the safety at the time of using it from points, such as a side effect, for a long period of time. This invention is made in view of the situation as above, and aims to let a side effect etc. offer the few high vascularization disease treatment agent of safety.
[0009]
[Means for Solving the Problem] Although the artificer of this invention etc. was inquiring about the biological activity of the lactoferrin which is food and which was milk-well isolated, and the hydrolyzate of those, he discovered the new fact that these matter checked the migration of the endothelial cell of the initial stage of the vascularization, and completed this invention.
[0010] That is, this invention offers the vascularization disease treatment agent which contains the peptide of the hydrolyzate origin of the hydrolyzate of lactoferrin and lactoferrin, and lactoferrin, the same or derivative of the peptides which have the amino acid sequence of homology, and these peptides as this peptide permitted pharmacologically, the salts of these peptides permitted pharmacologically, or such mixture as an active principle as what solves the above-mentioned technical problem.
[0011] Moreover, in the vascularization disease treatment agent of this invention, it requires also as a desirable mode that the content of the above-mentioned active principle is 0.1micro per 1g of tablets g-100mg, and that the above-mentioned peptide is a peptide which has the amino acid sequence indicated by either of the array number 1 to the array numbers 31. In addition, the target disorders [agent / vascularization disease treatment / of this invention] are the following disorders depended on for example, aforementioned Ito's definition.
a) Ophthalmology-disorder ** proliferative-diabetic-retinopathy ** retinopathy of prematurity (back lens fibroplasia)
** Explain the gestalt of implementation of this invention in detail below the unusual capillary network of the symptoms I chronic joint RIUMACHIU psoriasis E atherosclerosis nest outer

membrane which causes iris rubeosis ** sickle-cell-retinopathy ** vena-centralis-retinae atresia ** retinal-vein branching atresia ** central-artery-of-retina atresia ** senile-disciform-macular-degeneration ** and other ****.

[0012]

[Embodiments of the Invention] The lactoferrin used as an active principle of the vascularization disease treatment agent of this invention The appointment lactoferrin which removed iron from Lf(s) separated from commercial Lf, ****, and human milk by the conventional method, and these Lf(s) by the conventional method, It is the metal saturation the chelate of the metals, such as iron, copper, zinc, and manganese, was completely carried out [saturation] to appointment lactoferrin in part by the conventional method, or the general term of metal part saturation lactoferrin, and any one sort or two sorts or more of such mixture can be used.

[0013] The hydrolyzates of the lactoferrin used as an active principle of the vascularization disease treatment agent of this invention may be any of the things which refined the decomposition product which understood the aforementioned lactoferrin an added water part with the acid or the enzyme by the conventional method, and was obtained, and this hydrolyzate by the conventional method, or such mixture, and are the mixture of various peptides. Moreover, the hydrolyzate of lactoferrin is desirable especially as an active principle which unlike lactoferrin themselves there is no denaturation by heating, there is no loss of biological activity, and it uses for the vascularization disease treatment agent of this invention as compared with lactoferrin since the handling in a tablet-ized process is advantageous.

[0014] The peptides used as an active principle of the vascularization disease treatment agent of this invention are the peptide obtained from the decomposition product of the aforementioned lactoferrin by the well-known separation means, the same or derivative of the peptides which have the amino acid sequence of homology, and these peptides as this peptide permitted pharmacologically, the salts of these peptides permitted pharmacologically, or such arbitrary mixture, and can also be chemically compounded by the well-known method. These peptides can be obtained by the method indicated by each invention of JP,5-92994,A, JP,5-78392,A, JP,5-148297,A, JP,5-148296,A, and JP,5-148295,A.

[0015] The peptide obtained by the aforementioned method can illustrate the peptide which has the following amino acid sequence, its derivative, or salts as a desirable mode. For example, the peptide which has the amino acid sequence of the array numbers 1, 2, and 27, The salts or its derivative (JP,5-78392,A), the peptide that has the amino acid sequence of the array numbers 3, 4, 5, and 6, The salts or its derivative (JP,5-148297,A), the peptide that has the amino acid sequence of the array numbers 7, 8, 9, and 31, The salts or its derivative (JP,5-148296,A), the peptide that has the amino acid sequence of the array numbers 10-21, They are the salts or its derivative (JP,5-148295,A), the peptide that has the amino acid sequence of 26, 28, 29, and 30 from the array number 22, its salts, or its derivative (JP,5-92994,A).

[0016] As salts of the aforementioned peptide permitted pharmacologically, acid addition salts, such as a hydrochloride, phosphate, a sulfate, a citrate, a lactate, and a tartrate, can be illustrated, and the derivative which amidated or acylated the carboxyl group can be illustrated as a derivative of the aforementioned peptide permitted pharmacologically. Practical use can be presented with the vascularization disease treatment agent of this invention with the gestalt of the general physic tablet containing one sort of the active principle illustrated above, or two sorts or more. Moreover, according to the purpose of use, it is selectable suitably in various kinds of dosage forms, for example, medicines for external application, such as lotion, an aerosol agent (spray), liquefied paint, and an ointment, the ophthalmic solution, a suppository, a tablet, the pilule, powder, a capsule, the injection, etc. can be illustrated.

[0017] Although especially the loadings of the active principle of the vascularization disease treatment agent of this invention are not restricted but it can choose suitably according to the kind of disorder, a symptom, etc., the range of a desirable content is 0.1micro per 1g of tablets g-100mg. Moreover, since the active principle of the vascularization disease treatment agent of this invention is a natural product originating in food, it is clear. [satisfactory about those safeties]

[0018] Next, the example of an examination is shown and this invention is explained concretely. the example 1 of an examination -- using the cow aorta endothelial cell CPAE (from American Type Culture Collection to purchase), this examination was performed in order to investigate the effect of

the hydrolyzate of Lf to the migration of a cell, and lactoferrin, and the peptide of the hydrolyzate origin
(1) Cow lactoferrin of manufacture marketing of a sample (product made from OREOFINA.) Two sorts of peptides (sample 3) (sample 4) prepared, respectively by the same method as the hydrolyzate (sample 2) of the cow lactoferrin prepared by the same method as a sample 1 and the example 1 of reference, the example 2 of reference, and the example 3 of reference were used.
(2) The test-method CPAE cell was planted on the petri dish (diameter of 35mm), it cultivated by the Dulbecco strange method eagle culture medium (NISSUI PHARMACEUTICAL CO., LTD. make) which contains fetal calf serum 10%, and monochrome REA (monolayer) was made to form. Culture medium was removed, and a part of cell was exfoliated with the cutter knife, and it cultivated for 24 hours, respectively by this culture medium (contrast sample) of the amount of said which does not contain the 1ml Dulbecco strange method eagle culture medium (NISSUI PHARMACEUTICAL CO., LTD. make) and examination sample containing the examination sample (a sample 1 - sample 4) shown in Table 1. Formalin fixation of the after cell was carried out, and counting of the cell which carried out migration was carried out.
(3) an examination result -- the result of this examination is as being shown in Table 1 Those rates of each sample when setting the number of swarmers in a contrast sample to 100 show Table 1. Prevention activity [as opposed to the migration of an endothelial cell in the hydrolyzate (sample 2) of cow lactoferrin (sample 1) and lactoferrin and all of two sorts of peptides (samples 3 and 4)] was accepted. It was proved that having endothelial cell migration prevention activity is checked, and each of hydrolyzates of Lf and lactoferrin and peptides of the hydrolyzate origin prevents neogenesis of a vessel from this. In addition, although examined about other active principles, the almost same result was obtained.
[0019]
[Table 1]

添加量（μg/ml）	試料1	試料2	試料3	試料4
10	—	—	84	—
100	51	54	51	77
1000	62	—	—	52
10000	30	25	—	—
無添加（対照）	100	100	100	100

[0020] The example 1 (manufacture of the hydrolyzate of lactoferrin) of reference
Cow lactoferrin (product made from OREOFINA.) indicating it as Following bLf -- it is -- it was used and the hydrolyzate (it may be indicated as bLf-Hy below) of lactoferrin was prepared by the following method Commercial bLf500g was dissolved in 9.5l. of purified waters, the hydrochloric acid was added in the obtained solution, pH was adjusted to 3.0, 10g (Wako Pure Chem make) of pig pepsins of back marketing was added, and it hydrolyzed at 37 degrees C for 6 hours. Next, adjusted pH to 7.0 by the sodium hydroxide of 6 conventions, heat for 10 minutes at 80 degrees C, and the enzyme was made to deactivate, it cooled to the room temperature, cerite filtration was carried out, filtrate was freeze-dried, and about 470g of powdered lactoferrin hydrolyzates was obtained.
The example 2 (manufacture of the peptide from the hydrolyzate of lactoferrin) of reference
Cow lactoferrin (sigma company make) 50mg of marketing was dissolved in 0.9ml of purified waters, pH was adjusted to 2.5 with the hydrochloric acid of 0.1 conventions, pig pepsin (sigma company make) 1mg of back marketing was added, and it hydrolyzed at 37 degrees C for 6 hours. Subsequently, adjusted pH to 7.0 by the sodium hydroxide of 0.1 conventions, and heated for 10 minutes at 80 degrees C, the enzyme was made to deactivate, it cooled to the room temperature, at-long-intervals heart separation was carried out by 15,000rpm for 30 minutes, and the transparent supernatant liquid was obtained. The vacuum drying of the fraction which applies 100micro of this supernatant liquid l to the high performance chromatography using TSK gel ODS-120T (TOSOH CORP. make), is eluted in 20% acetonitrile which contains TFA (trifluoroacetic acid) 0.05% for 10 minutes after sample pouring by the 0.8ml rate of flow for /, is eluted in the gradient of 20 - 60% of acetonitrile which contains TFA 0.05% for 30 minutes the back, and is eluted in 24 - 25 minutes was collected and carried out. The fractions which dissolve this dry matter in a purified water by 2% (W/V) of concentration, are missing from the high performance chromatography using TSK gel

ODS-120T (TOSOH CORP. make) again, are eluted in 24% acetonitrile which contains TFA 0.05% for 10 minutes after sample pouring by the 0.8ml rate of flow for /, are eluted in the gradient of 24-32% of acetonitrile which contains TFA 0.05% for 30 minutes the back, and are eluted in 33.5 - 35.5 minutes were collected. The above-mentioned operation was repeated 25 times, and carried out the vacuum drying, and peptide about 1.5mg was obtained.

[0021] It understood an added water part with the hydrochloric acid of 6 conventions of the above-mentioned peptide, and the amino acid composition was analyzed by the conventional method using the amino acid analyzer. 25 times of Edman degradation was performed for the same sample using gaseous-phase seeking ENSA - (applied biotechnology systems company make), and the array of 25 amino acid residues was determined. Moreover, it checked that a disulfide bond existed by the disulfide-bond analysis method [Analytical Biochemistry (Analytical Biochemistry), the 67th volume, the 493rd page, and 1975] using DTNB [a 5 and 5-dithio-screw (2-nitroglycerine benzoic acid)].

[0022] Consequently, having the amino acid sequence of a publication for the array number 26 which this peptide consisted of 25 amino acid residues, the 3rd and the 20th cysteine residue carried out the disulfide bond, two amino acid residues combined with the N terminus side from the 3rd cysteine residue, and five amino acid combined with C-end side from the 20th cysteine residue, respectively was checked.

The example 3 (composition of a peptide) of reference

Peptide automatic synthesizer unit (Pharmacia LKB Biotechnology, Inc. make.) Based on the solid phase peptide synthesis method [journal OBU chemical society Perkin I (Journal of Chemical Society Perkin I), the 538th page, and 1981] by German shepherd etc., the peptide was compounded as follows using LKBBiolynx4170.

[0023] Amino acid [less-or-equal Fmoc-amino acid or Fmoc which protected the amine functional group by 9-fluorenyl methoxycarbonyl group - Added N and N-dicyclohexylcarbodiimide to] which may be indicated to be the name (for example, Fmoc-asparagine) of peculiar amino acid, it was made to generate the anhydride of desired amino acid, and this Fmoc-amino acid anhydride was used for composition. In order to manufacture a peptide chain, the Fmoc-asparagine anhydride equivalent to the asparagine residue of C-end is fixed to a URUTOROSHIN A resin (Pharmacia LKB Biotechnology, Inc. make) by making a dimethylamino pyridine into a catalyst through the carboxyl group. Subsequently, this resin is washed by the dimethylformamide containing a piperidine, and the protective group of the amine functional group of C-end amino acid is removed. Distributor shaft coupling of the Fmoc-arginine (Pmc:2, 2, 5 and 7, 8-Pentamethylchroman-6-sulphonyl basis) anhydride which is equivalent to the 2nd from C-end of an after amino acid sequence was carried out to the deprotection amine functional group of the asparagine fixed to the resin through the aforementioned C-end amino acid residue. The glutamine, the tryptophan, the glutamine, and the phenylalanine were fixed one by one like the following. After distributor shaft coupling of all amino acid was completed and the peptide chain of a desired amino acid sequence was formed, TFA, 5% phenol, and the solvent that consists of an ethanediol 1% performed removal of a protective group, and desorption of a peptide 94%, the high performance chromatography refined the peptide, this solution was condensed, it dried and peptide powder was obtained.

[0024] The amino acid composition was analyzed by the conventional method using the amino acid analyzer about the aforementioned peptide, and it checked having the amino acid sequence of a publication for the array number 10. Next, although an example is shown and this invention is explained still more concretely, this invention is not limited to the following examples.

[0025]

[Example]

Example 1 (hydrophilic ointment)

Commercial lactoferrin (product made from OREOFINA) 10 (g)

A white vaseline 250 Stearyl alcohol 220 Propylene glycol 120 URARIRU sodium sulfate 15 Methyl parahydroxybenzoate 0.25 Propylparaben 0.15 Purified water Optimum dose conventional method. Each aforementioned component was mixed and 1000g hydrophilic ointment was manufactured. All the raw materials used commercial elegance.

Example 2 (tablet)

Peptide obtained from the lactoferrin hydrolyzate (it manufactures by the same method as the example 2 of reference) 15 (mg)
A crystalline cellulose 170 Corn starch 66 Talc 11 Magnesium stearate It is a conventional method about each raw material of the aforementioned rate per 31 locks. It corned, and it dried, and it mixes uniformly and the tablet was obtained [it tableted and]. In addition, each raw material other than the peptide obtained from the lactoferrin hydrolyzate used commercial elegance.
Example 3 (powder)
Hydrolyzate of lactoferrin (it manufactures by the same method as the example 1 of reference) 50 (g)

Crystalline cellulose 375 Corn starch 575 aforementioned each material was mixed uniformly and 1000 bags of powder was prepared by the conventional method. In addition, each raw material other than the hydrolyzate of lactoferrin used commercial elegance.
Example 4 (capsule)
Synthetic peptide originating in a lactoferrin hydrolyzate (it manufactures by the same method as the example 3 of reference) 10 (mg)
A lactose 120 Crystalline cellulose 42 Carboxymethyl cellulose 10 Talc 15 Magnesium stearate It is a conventional method about each raw material of the aforementioned rate per 31 locks. It mixed uniformly and the capsule was prepared using the encapsulation machine. In addition, each raw material other than the synthetic peptide originating in a lactoferrin hydrolyzate used commercial elegance.
Example 5 (applying-eyewash agent)
Synthetic peptide originating in a lactoferrin hydrolyzate (it manufactures by the same method as the example 3 of reference) 10 (mg)
Propylene glycol 500 Disodium hydrogenphosphate 10 Boric acid 1300 Sodium chloride Each raw material of the rate of the rate of the 900 above. It dissolved in 100ml of purified waters, and through and the applying-eyewash agent were prepared for the sterilization filter. In addition, each raw material other than the synthetic peptide originating in a lactoferrin hydrolyzate used commercial elegance.
[0026]
[Effect of the Invention] There is no side effect etc., and when it is used for a long period of time, the blood vessel new disease medical treatment agent which is satisfactory about safety is ****(ed) by this invention as explained in detail above. New possibility is brought to the medical treatment of intractable diseases, such as an ophthalmology-disease group, rheumatoid arthritis, psoriasis, and an unusual capillary network of an atherosclerosis nest outer membrane, by this.
[0027]
[Layout Table]
array number: -- length [of one array]: -- mold [of 11 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0028]
array: -- Lys R01 R01 R01 R01 Gln R01 R01 Met Lys Lys1 5 10 array number: -- length [of two arrays]: -- mold [of 11 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0029] array: -- Lys R01 R01 R01 R01 Gln R01 R01 Met Arg Lys1 5 10 array number: -- length [of three arrays]: -- mold [of six arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0030] array: -- Arg R01 R01 R01 R01 Arg1 5 array number: -- length [of four arrays]: -- mold [of six arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.

[0031] array: -- Lys R01 R01 R01 R01 Arg1 5 array number: -- length [of five arrays]: -- mold [of six arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0032] array: -- Lys R01 R01 R01 R01 Lys1 5 array number: -- length [of six arrays]: -- mold [of six arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0033] array: -- Arg R01 R01 R01 R01 Lys1 5 array number: -- length [of seven arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0034] array: -- Arg R01 R01 R01 Arg1 5 array number: -- length [of eight arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0035] array: -- Lys R01 R01 R01 Arg1 5 array number: -- length [of nine arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0036] array: -- Arg R01 R01 R01 Lys1 5 array number: -- length [of ten arrays]: -- mold [of six arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation
[0037] array [:P] he Gln Trp Gln Arg Asn1 5 array number: -- length [of 11 arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation
[0038] array [:P] he Gln Trp Gln Arg1 5 array number: -- length [of 12 arrays]: -- mold [of four arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation
[0039] array: -- Gln Trp Gln Arg1 array number: -- length [of 13 arrays]: -- mold [of three arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation
[0040] array: -- Trp Gln Arg1 array number: -- length [of 14 arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation
[0041] array: -- Arg Arg Trp Gln Trp1 5 array number: -- length [of 15 arrays]: -- mold [of four arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation
[0042] array: -- Arg Arg Trp Gln1 array number: -- length [of 16 arrays]: -- mold [of four arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation
[0043] array: -- Trp Gln Trp Arg1 array number: -- length [of 17 arrays]: -- mold [of three arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation
[0044] array: -- Gln Trp Arg1 array number: -- length [of 18 arrays]: -- mold [of six arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation
[0045] array: -- Leu Arg Trp Gln Asn Asp1 5 array number: -- length [of 19 arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation
[0046] array: -- Leu Arg Trp Gln Asn1 5 array number: -- length [of 20 arrays]: -- mold [of four arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation
[0047] array: -- Leu Arg Trp Gln1 array number: -- length [of 21 arrays]: -- mold [of three arrays]:

-- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation
[0048] array: -- Arg Trp Gln1 array number: -- length [of 22 arrays]: -- mold [of 20 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and the peptide which contains this peptide as fragmentation It sets in the following array and they are No. 2. Cys and No. 19 Cys is carrying out the disulfide bond.
[0049]
Array: Lys Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro 1 5 10 15 Ser Ile Thr Cys Val 20 array number: -- length [of 23 arrays]: -- mold [of 20 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. In order that Cys* may prevent formation of a disulfide bond in the following array, the cysteine which embellished the thiol group chemically is shown.
[0050]
Array: Lys Cys* Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro 1 5 10 15 Ser Ile Thr Cys* Val 20 array number: -- length [of 24 arrays]: -- mold [of 20 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. It sets in the following array and they are No. 2. Cys and No. 19 Cys is carrying out the disulfide bond.
[0051]
Array: Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg Gly Pro 1 5 10 15 Pro Val Ser Cys Ile 20 array number: -- length [of 25 arrays]: -- mold [of 20 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. In order that Cys* may prevent formation of a disulfide bond in the following array, the cysteine which embellished the thiol group chemically is shown.
[0052]
Array: Lys Cys* Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg Gly Pro 1 5 10 15 Pro Val Ser Cys* Ile 20 array number: -- length [of 26 arrays]: -- mold [of 25 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. It sets in the following array and they are No. 3. Cys and No. 20 Cys is carrying out the disulfide bond.
[0053]
Array: Phe Lys Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala 1 5 10 15 Pro Ser Ile Thr Cys Val Arg Arg Ala Phe 20 25 array number: -- length [of 27 arrays]: -- mold [of 11 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide.
[0054] array: -- Lys Thr Arg Arg Trp Gln Trp Arg Met Lys Lys1 5 10 array number: -- length [of 28 arrays]: -- mold [of 38 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- this peptide and peptide which contains this peptide as fragmentation It sets in the following array and they are No. 16. Cys and No. 33 Cys is carrying out the disulfide bond.
[0055]
Array: Lys Asn Val Arg Trp Cys Thr Ile Ser Gln Pro Glu Trp Phe Lys 1 5 10 15 Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro Ser 20 25 30 Ile Thr Cys Val Arg Arg Ala Phe 35 array number: -- length [of 29 arrays]: -- mold [of 32 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. It sets in the following array and they are No. 10. Cys and No. 27 Cys is carrying out the disulfide bond.
[0056]
Array: Thr Ile Ser Gln Pro Glu Trp Phe Lys Cys Arg Arg Trp Gln Trp 1 5 10 15 Arg Met Lys Lys Leu Gly Ala Pro Ser Ile Thr Cys Val Arg Arg 20 25 30 Ala Phe array number: -- length [of 30 arrays]: -- mold [of 47 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. In the following array, it is the length 36 of an array, and they are No. 9, No. 26, and No. 35. No. 9 of the peptide which has Cys Cys and No. 26 Cys carries out a disulfide bond and they are No.

35 of the peptide of the length 36 of the above-mentioned array. No. 10 of the peptide to which Cys is the length 11 of an array and has Cys in No. 10 Cys is carrying out the disulfide bond.
[0057]
Array: Val Ser Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn 1 5 10 15 Met Arg Lys Val Arg Gly Pro Pro Val Ser Cys Ile Lys Arg Asp 20 25 30 Ser Pro Ile Gln Cys Ile 35 Gly Arg Arg Arg Arg Ser Val Gln Trp Cys Ala 1 5 10 array number: -- length [of 31 arrays]: -- mold [of five arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- feature [of a peptide array]: -- let this peptide and this peptide be fragmentation The included peptide. it sets in the following array -- R01 The arbitrary amino acid residues except Cys are shown.
[0058] Array: Lys R01 R01 R01 Lys 1 5.

[Translation done.]